# THE OXYGEN CONSUMPTION OF THE CRAYFISH ORCONECTES IMMUNIS AND ORCONECTES NAIS IN RESPONSE TO TEMPERATURE AND TO OXYGEN SATURATION

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IN CONTEMPORARY biology there has developed an increasing awareness of the value of physiological studies in answering some of the intriguing problems of speciation, ecological distribution, and evolution. Significant relationships have been shown between major and/or minor groups of animals through the study of such phenomena as ionic concentrations of the blood, blood pigments, phosphagens, and nitrogenous excretions (Baldwin, 1949). Also, the development of a variety of physiological mechanisms has allowed closely related animals to distribute themselves into habitats that are different in certain qualities. Although morphological differences are present, it is often physiological differentiation which has allowed radiation and eventual speciation to occur in many animals. Prosser (1955) aptly expressed the position which physiological studies need to take:

> The selective value of new genetic patterns depends on functional adaptation, and sometimes the morphological characters which are useful in systematics may not be necessarily adaptive in themselves, but may be outward signs, associated by linkage or pleiotropism with subtle physiological characters. . . . Comparison of physiological adaptations should contribute much to an understanding of interspecific relations, intraspecific variation, and the bases for ecological ranges.

In its broadest sense, the study presented here was made with the hope that it might contribute to increased understanding in the areas mentioned above.

Interest in this study was stimulated by the local ecological distribution of two species of the genus *Orconectes*. The species *O. immunis* (Hagen) is found in temporary roadside ditches southeast of Lawrence, in Douglas County, Kansas; *O. nais* (Faxon) is a common inhabitant of running streams and permanent ponds in the same area. Either species is rarely found in association with the other. Each is apparently limited to a particular habitat notably different from that of the other.

In the local situation studied, among many possible ecological variations, differences in temperature, dissolved oxygen concentration, and presence or absence of open water seem to be important. The metabolic rates of crayfish are vitally affected by changes in these conditions. Hence, if the species are differentially adapted to such ecological conditions, one of the best means of detecting these adaptations is by determining the oxygen consumption of each species under such varying conditions as the organisms might encounter in the natural environment.

Bovbjerg (1952) found behavioral and physiological differences between crayfish from temporary ponds and from streams in the Chicago area. Under experimental conditions, the pond species, *Cambarus fodiens*, was more resistant to desiccation, less tolerant of flowing water, and more tolerant of increased tem-

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perature and decreased oxygen concentration than was *O. propinquus*, a species living in streams. However, he judged that the difference in lethal temperatures is not ecologically limiting, since the difference occurs above environmental temperatures. Park, Gregg, and Lutherman (1940) and Park (1945) tested several species of crayfish for their toleration of reduced oxygen concentration and increased temperature. Pond species lived 50 per cent longer, reduced the oxygen level lower, and were more resistant to heat than stream species, under the conditions of the experiment.

In another study of toleration of reduced oxygen saturation, Burbank, Edwards, and Burbank (1948) found that cave crayfish, *Cambarus setosus*, tolerated reduced oxygen saturation better than did crayfish living in open streams. They concluded that the cave crayfish has a lower metabolic rate and so reduces the oxygen level less rapidly.

Metabolic differences correlated with differences in habitat have been found in species of *Astacus*. The species *A. leptodactylus*, a lake dweller, has a lower rate of metabolism than *A. torrentium*, which lives in swift streams (Prosser *et al.*, 1950).

Among other arthropods, studies of metabolic rates of certain insect larvae (including mayflies, caddisflies, ephemerids, and chironomids) and of isopods reveal higher metabolic rates and less toleration of anoxia in stream inhabitants than in pond-dwelling kinds (Fox and Simmonds, 1933; Fox, Simmonds, and Washburn, 1935; Walshe, 1948).

In research on decapods, physiological adaptation to various environments has been studied largely from two points of view. One group of studies has used the criterion of toleration or survival under a particular set of conditions, indicating adaptive capacity but without quantitative measurements of metabolic rates. A second body of literature reports the desired quantitative measurements of oxygen consumption as an indicator of metabolic rate, but studies have been limited either to the effects of temperature on metabolism at saturated oxygen levels or to the effects of reduced oxygen saturation at one temperature.

Therefore, this study was designed to determine the responses to changes in temperature at different oxygen (saturation) levels within the physiological range and the responses to changes in oxygen saturation at different temperatures in the biokinetic zone. The purpose of this study is to elucidate the physiological dynamics that are important in determining the ecological distribution of the two species of crayfish, *O. immunis* and *O. nais*.

All experimental work was done in the laboratory of aquatic biology of the department of zoölogy, University of Kansas, Lawrence, Kansas. The authors wish to thank Mr. Gunther Schlager for assistance with the statistical treatment of the data.

## MATERIAL AND METHODS

The three experimental variants selected were the temperature and oxygen saturation of the ambient water and the body weight of the animals. The four temperatures at which rates of oxygen consumption were determined were 16°, 24°, 30°, and 35° C. The four experimental levels of oxygen saturation chosen were full, one-half, one-quarter, and one-eighth saturation at the respective temperatures. Three divisions of the entire size range of the populations were made, setting arbitrary boundaries between the small (average weight, 1.80 gm.), mid (average weight, 3.45 gm.), and large animals (average weight, 8.37 gm.).

Oxygen consumption is believed or

known to vary with a number of other factors including sex, diet, season, and activity. These factors were either treated randomly or controlled, so that their effect was negligible.

All the crayfish used in the testing were seined in an area with a radius of three and one-half miles in the valley of the Wakarusa River, south of Lawrence, Douglas County, Kansas.

Oxygen consumption was determined by means of the flow method similar to that used by van Weel, Randall, and Takata (1954). Preliminary investigations indicated that one and one-half days was sufficient time for the crayfish to stabilize their metabolism at a new temperature. Therefore, determinations of oxygen consumption were made after animals had acclimated for that period of time.

The temperature of the laboratory was regularly checked with a thermistor thermometer during the two-day period before any experiment. Variation for any one temperature level was less than 2° C. The oxygen concentration of the supply carboy was determined prior to the start of each experiment. Actual oxygen concentrations varied within 5 per cent of stated saturation levels. Each crayfish was examined before an experiment and used only if in good physical condition. The absence of three or more walking legs was considered evidence of poor physical condition. No crayfish judged to be in the process of molting were used.

Transference of the crayfish from an aquarium to the respiratory cylinder disturbed them somewhat, tending to cause a temporary increase in metabolic rate. Because of this, twenty to thirty minutes were allowed for the animals to adjust to the new environment before the water sample was taken.

Jordan and Guittart (1938), as cited in van Weel, Randall, and Takata (1954), reported that artificial immobilization "markedly affects" oxygen consumption in *Astacus fluviatilis*. In this study, however, even the largest animals were able to move about freely; yet all crayfish typically become inactive soon after being put into the respiratory cylinders. Berg, Lumbye, and Ockelman (1958) reported that shaking the respiratory cylinders resulted in increased oxygen consumption; thus any movement of the cylinders was avoided when crayfish were in them.

Inasmuch as crayfish were found to be less active in the dark than in the light in the laboratory, all experiments were made with cylinders covered with a heavy brown cloth, reducing light intensity and reducing the effects of the movements of the worker about the room. The twenty- to thirty-minute period of adjustment of the animal, mentioned above, was considered sufficient time to allow the crayfish to acclimate their oxygen consumption to the new oxygen level. Van Weel, Randall, and Takata (1954) used a thirty-minute acclimation time in controlled experiments on Pacific decapods of weights comparable to those of animals used in this study.

After the water samples for oxygen determination had been taken, the animals were blotted with a soft cloth to remove excess moisture and were weighed on an analytical balance with accuracy of 0.01 gm. After being weighed, the crayfish were returned to the aquaria.

The oxygen consumption of five animals of each species could be determined simultaneously. Usually the oxygen consumption of three such sets of five animals per species was determined in the course of a dawn or dusk period of two and one-half hours. The dawn and dusk periods corresponded to the peaks in the diurnal rhythm of oxygen consump-

tion of both species.

The oxygen content of the water sample taken by the flow method was determined by means of the unmodified Winkler method (Standard Methods, 1955). When the unmodified method and the Rideal-Stewart permanganate modification of the Winkler method (Welch, 1948) were used on duplicate water samples, no difference in oxygen content was found between the samples. The variation between duplicate samples of water tested for dissolved oxygen content was less than 0.1 mg/l.

TABLE 1

MEAN OXYGEN-CONSUMPTION RATE (CC/GM/HR) AND NUMBER OF ANIMALS TESTED

| Animal Size | Oxygen Saturation | Species | Temperature (° C.) 16 | 24 | 30 | 35 |
|---|---|---|---|---|---|---|
| Small | Full | *immunis* | 16/.044* | 14/.139 | 15/.212 | 12/.179 |
| | | *nais* | 13/.054 | 20/.137 | 17/.184 | 10/.153 |
| | ½ | *immunis* | 17/.067 | 17/.084 | 19/.109 | 27/.181 |
| | | *nais* | 13/.055 | 15/.088 | 16/.127 | 32/.171 |
| | ¼ | *immunis* | 15/.043 | 16/.078 | 35/.079 | 14/.097 |
| | | *nais* | 14/.039 | 18/.071 | 34/.070 | 14/.076 |
| Mid | Full | *immunis* | 19/.109 | 15/.124 | 10/.129 | 24/.126 |
| | | *nais* | 10/.116 | 15/.123 | 10/.126 | 18/.102 |
| | ½ | *immunis* | 12/.064 | 28/.105 | 31/.114 | 21/.155 |
| | | *nais* | 13/.075 | 28/.089 | 28/.125 | 24/.148 |
| | ¼ | *immunis* | 15/.050 | 19/.065 | 16/.078 | 9/.103 |
| | | *nais* | 14/.047 | 21/.068 | 18/.083 | 13/.085 |
| Large | Full | *immunis* | 14/.068 | 26/.113 | 15/.124 | 12/.113 |
| | | *nais* | 15/.066 | 23/.110 | 15/.114 | 12/.109 |
| | ½ | *immunis* | 15/.067 | 14/.082 | 14/.109 | 15/.128 |
| | | *nais* | 15/.057 | 14/.078 | 14/.112 | 12/.111 |
| | ¼ | *immunis* | 15/.040 | 24/.053 | 25/.064 | 24/.064 |
| | | *nais* | 15/.038 | 24/.049 | 23/.057 | 22/.055 |
| | ⅛ | *immunis* | 10/.029 | 9/.036 | 6/.044 | 6/.052 |
| | | *nais* | 9/.030 | 8/.027 | 8/.034 | 6/.030 |

* Number of animals tested precedes mean oxygen-consumption rate.

## EFFECT ON OXYGEN CONSUMPTION OF VARIANTS

A summary of number of animals tested and the mean oxygen consumption (cc/gm/hr) for each temperature, oxygen saturation, and size of both species is recorded in Table 1. Through use of a factorial analysis (Snedecor, 1955), the effect of the variants acting independently and the effect of any interactions between variants were determined. Because of the nature of the factorial analysis, the one-eighth saturation rates were not included in that particular statistical procedure.

### TEMPERATURE

Temperature markedly affected the oxygen consumption of both species (Table 2); generally, oxygen consumption increased significantly with an increase in temperature. The rate/temperature ($R/T$) curves for large animals of *O. immunis* (Fig. 1) also illustrate the general character of the $R/T$ curves of the mid and small animals. The $R/T$ curve for *O. nais* is similar to that of *O. immunis*, except under certain conditions to be discussed in a later section.

The calculation of regression coeffi-

cients revealed that a true linear regression line fits the values plotted at the one-eighth and one-half saturated levels, indicating that the log of oxygen consumption varies linearly with temperature. Thus at a low rate of oxygen consumption a unit increase in temperature affects the metabolic rate more than at a high rate of oxygen consumption. Two exceptions to the linear relationship are seen in the saturated and one-quarter saturated curves. The rate at full oxygen saturation at 37° C. is depressed because it was taken 2° C. higher than the other rates representing the highest temperature level. This was the first saturation level at which animals were tested. It was concluded that at 37° C. new variables associated with the thermal death limit were affecting the metabolic rate. The highest temperature at which animals were tested was changed to 35° C. for the remaining rate determinations.

TABLE 2

THE *f*-VALUES OF FACTORIAL ANALYSIS OF DATA PRESENTED IN TABLE 1 (DATA AT ONE-EIGHTH OXYGEN SATURATION OMITTED)

| | Species | |
|---|---|---|
| | *immunis* | *nais* |
| *Main effects:* | | |
| Size | 4.5* | 5.7* |
| Saturation | 24.3** | 35.0** |
| Temperature | 10.8** | 18.6** |
| *Interactions:* | | |
| Size and Saturation | 0.6 | 0.5 |
| Size and Temperature | 1.5 | 1.7 |
| Saturation and Temperature | 2.0 | 2.5 |

The rate at which oxygen consumption increases in relation to temperature is expressed as $Q_{10}$, according to the equation of van't Hoff. The $Q_{10}$ was of some value in comparing the four separate lines plotted in Figure 1. Omitting the values determined at 35° and 37° C. for all four saturation levels because of their inconsistency, the $Q_{10}$ values were calculated with the following results: saturated, 16–31° C., $Q_{10} = 1.49$; one half saturated, 16–30° C., $Q_{10} = 1.43$; one-quarter saturated, 16–30° C., $Q_{10} = 1.40$; one-eighth saturated, 16–30° C., $Q_{10} = 1.34$. The values of $Q_{10}$ at different oxygen saturations were similar, with a slight decrease in $Q_{10}$ noted with reduced saturations. These results, as well as the results of the factorial analysis, indicate that the $R/T$ curve of *O. immunis* was not affected greatly by changes in oxygen saturation. Essentially, the $R/T$ curves are translations either higher or lower on the graph at different oxygen saturations.

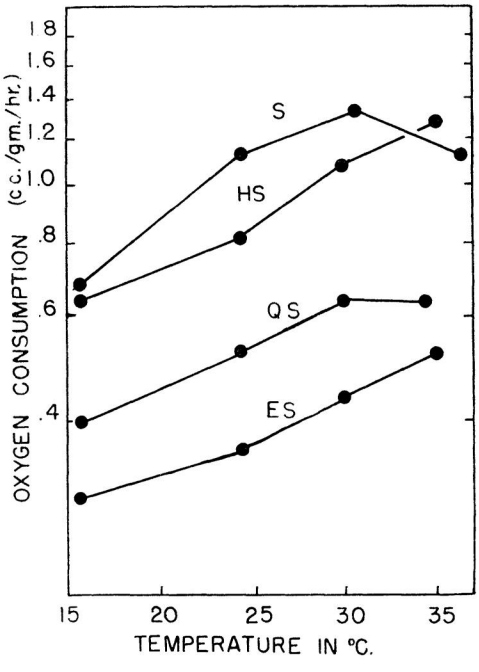


FIG. 1.—The relation between oxygen consumption of large *O. immunis* and temperature at different oxygen concentrations. Oxygen concentration is represented by *S*, saturated; *HS*, one-half saturated; *QS*, one-quarter saturated; *ES*, one-eighth saturated.

OXYGEN CONCENTRATION

Factorial analysis revealed that the level of concentration of dissolved oxygen affected the oxygen consumption of both species (Table 2); there was a significant

decrease in oxygen consumption as the oxygen concentration decreased. The relationship between oxygen concentration and the rate of oxygen consumption of large individuals of *O. immunis* is shown in Figure 2. The four points for each temperature curve in Figure 2 are the

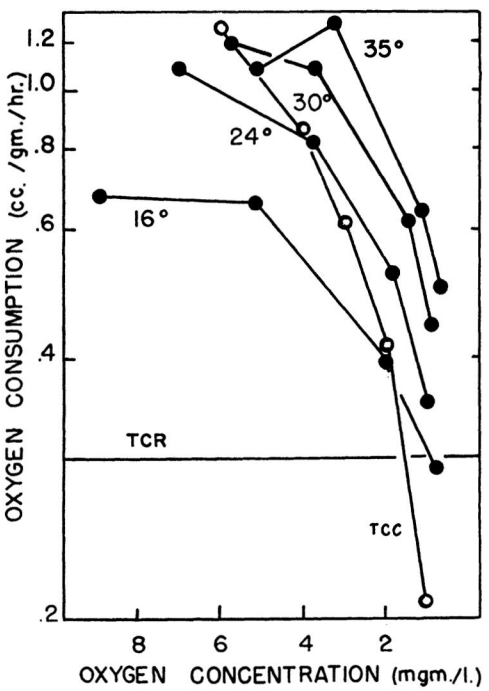


FIG. 2.—The relation between oxygen consumption of large *O. immunis* and oxygen concentration at different temperatures. *TCR* indicates the theoretical curve of a regulator; *TCC* indicates the theoretical curve of a conformer. The point at full saturation on the 35° C. curve was determined at 37° C.

four levels of oxygen concentrations corresponding to full, one-half, one-quarter, and one-eighth saturation. As an aid in visualizing how much an animal regulates or conforms under these experimental conditions, two theoretical curves, that of a conformer (*TCC*) and that of a regulator (*TCR*), are also plotted in Figure 2. The *TCC* is the same as a straight-line plot on a double-linear graph, showing a 1:1 relationship. This illustrates that for a unit reduction in saturation level, a corresponding unit reduction in metabolic rate will result. When plotted on semilog coordinates, the straight line becomes curved (Fig. 2). The *TCR* denotes that through any change in oxygen concentration, the metabolic rate will remain constant, as the line shows. The general character of the response to reduction of oxygen concentration is a reduction of metabolic rate. This was evident at all temperatures. None of the curves clearly follow the theoretical curves of a regulator or a conformer. At each temperature the metabolic-rate curve is intermediate between them, that is to say, under this range of conditions the animals partially regulate their internal states, but the degree of regulation decreases as the temperature increases. The curve at 16° C. more nearly approaches the *TCR* than that of any other temperature. The curve from 8.8 mg/l to 5.1 mg/l is nearly a simple translation of the *TCR*, definitely indicating that the animals were regulating. The curve of the three points determined at 35° C. is more nearly like the theoretical conformer curve than any of the other four temperature curves. The data show that the degree to which an animal regulates is not only dependent on the oxygen saturation but is also a function of temperature. There is a tendency for rotation of the curves in a clockwise direction as temperature increases. The meeting point of all four curves must necessarily be between 1 and 0 mg. of oxygen/l.

A different expression of metabolic phenomena is made possible by calculating the per cent utilization of oxygen/gm of body weight. A summary of the mean per cent of oxygen utilization/gm of large animals of both species for each temperature and saturation is recorded in Table 3.

The per cent of utilization of oxygen/gm of large *O. immunis* is plotted against oxygen saturation in Figure 3. The *TCC* and *TCR* were derived in the same way as were those in Figure 2, except that in this graph, the perfect regulator, with a constant oxygen consumption, uniformly increases its per cent of utilization. The perfect conformer here utilizes the same per cent of oxygen as the oxygen concentration decreases, resulting in a steadily decreasing metabolism.

Metabolic levels at each temperature are distinctly shown in Figure 3. At all temperatures, with each successive decrease in oxygen concentration, the per cent of utilization per gram body weight increases in most instances. Even at 35° C., the curve indicates that the animals tend to regulate their oxygen withdrawal at low concentrations. This is unexpected because the same conditions, plotted as oxygen-consumption rate in Figure 2, show that the animals are closely conforming to the environmental condition. The comparison between the curves in the two figures indicates that it is possible for crayfish to increase the per cent of oxygen withdrawn from the water and still have a decreasing metabolic rate.

The responses to oxygen saturation have been studied in a number of decapods. Amberson, Mayerson, and Scott (1925) found the American lobster, *Homarus americanus*, to conform its metabolic rate to the level of oxygen tension, as does *H. vulgaris* on the European side of the Atlantic Ocean (Thomas, 1954).

Maloeuf (1936) studied *Cambarus bartoni* and found essentially a conforming type of metabolic rate. Helff (1928), on the other hand, reports *Cambarus* (*Orconectes*) *immunis* to regulate its metabolism down to 20–40 per cent of saturated oxygen at 25° C.

TABLE 3

MEAN PER CENT OF UTILIZATION OF OXYGEN PER GRAM OF BODY WEIGHT

| Animal Size | Saturation Level | Species | Temperature (° C.) 10 | 24 | 30 | 35 |
|---|---|---|---|---|---|---|
| Large | Full | *immunis* | 1.40 | 2.45 | 3.15 | 2.95 |
| | | *nais* | 1.00 | 2.76 | 3.62 | 3.64 |
| | 1/2 | *immunis* | 1.89 | 3.17 | 3.79 | 5.37 |
| | | *nais* | 1.75 | 3.15 | 3.25 | 4.23 |
| | 1/4 | *immunis* | 2.88 | 3.90 | 5.61 | 6.28 |
| | | *nais* | 3.64 | 3.47 | 5.08 | 5.45 |
| | 1/8 | *immunis* | 4.94 | 4.97 | 5.27 | 7.82 |
| | | *nais* | 5.56 | 4.01 | 4.18 | 4.89 |

Hiestand (1931) analyzed the respiratory dynamics of *Cambarus virilis*, finding it able to regulate its metabolic activities down to 2.84 mg. of $O_2$/l. (about 40 per cent saturated), if given sufficient time to acclimate. If sufficient time were not given, it was possible to impose conformity of metabolic rate to the environment by the experimental conditions.

Van Weel, Randall, and Takata (1954), measuring metabolic rates in terms of oxygen utilization, found correlations between habitat and ability to regulate oxygen withdrawal in seven species of Hawaiian marine decapods. Inhabitants of mud flats apparently regulate over a wide range of oxygen concentrations, whereas inhabitants of near-terrestrial and well-aerated tidal zones

show regulatory activity only under relatively low oxygen-concentration levels.

BODY WEIGHT

Factorial analysis (Table 2) indicated a significant correlation between weight and metabolic rate (Fig. 4). The curves in the figure are eye-fitted from mean rates of the three size groups of animals at oxygen-concentration levels from saturation to one-quarter. The results indicate an inverse relationship between body weight and metabolic rate, for as body weight increases, oxygen consumption/gm/hr decreases. There is some variation in the slope of the lines at different temperatures, but no significance is attributed to it. A detailed study of the relationship of metabolic rate to body weight was not feasible because of the difficulty in accurately obtaining the oxygen-consumption rates of the small animals. At times the amount of oxygen removed from the water by the small animals was so slight at low temperatures that it was within the range of experimental error.

INTERACTIONS

The statistical analysis of the combined effects of variants (Table 2) indicates that none of the possible combinations of variants are significant at the 5 per cent level in either species. Evidently the individual variants, as they operate independently of each other, have a more pronounced and obvious effect on metabolic rate than do interacting variants.

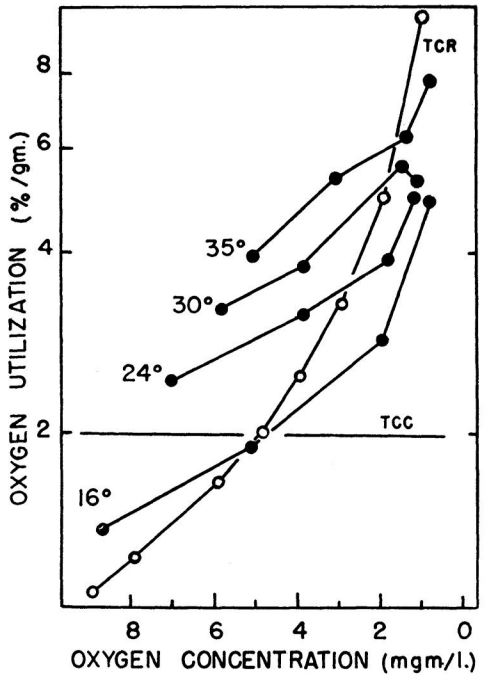


FIG. 3.—The relation between per cent utilization of oxygen per gram of body weight of large *O. immunis* and oxygen concentration at different temperatures. *TCR* indicates the theoretical curve of a regulator; *TCC* indicates the theoretical curve of a conformer.

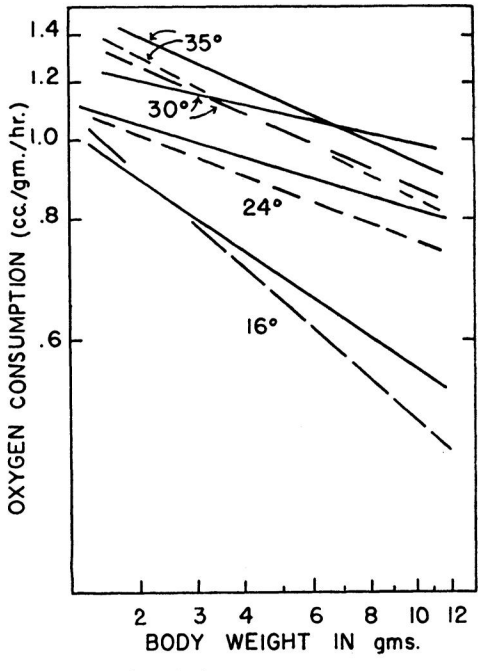


FIG. 4.—The relation between oxygen consumption and body weight at different temperatures for *O. immunis* (——) and *O. nais* (- - -). Metabolic rates at oxygen levels of saturation, one-half saturation, and one-quarter saturation are averaged for each temperature. Curves are eye-fitted from three points representing the mean oxygen consumption of the three size groups. The rates at 37° C. at full oxygen saturation and the rates of the small animals at 16° C. are omitted from the curves.

The size-saturation interaction *f*-value is far from significant even at the 5 per cent level, as is the size-temperature value, although the latter value is somewhat closer to statistical significance. That these *f*-values are quite low might have been expected because it has been shown that the effect of size acting independently was significant only at the 5 per cent level. The saturation-temperature interaction *f*-values also fall short of statistical significance; however, they

TABLE 4

THE *f*-VALUES OF ANALYSIS OF VARIANCE, COMPARING RESPONSES OF TWO SPECIES TO TEMPERATURE AT SELECTED LEVELS OF OXYGEN SATURATION

| Animal Size | Saturation Level | *f*-Value |
|---|---|---|
| Large | Full | <1.0 |
| | 1/2 | 1.6 |
| | 1/4 | 4.6* |
| | 1/8 | 7.5** |
| Mid | Full | <1.0 |
| | 1/2 | <1.0 |
| | 1/4 | <1.0 |
| Small | 1/2 | <1.0 |
| | 1/4 | 6.7* |

TABLE 5

THE *f*-VALUES OF ANALYSIS OF VARIANCE COMPARING RESPONSES OF TWO SPECIES TO OXYGEN-CONCENTRATION LEVEL AT SELECTED TEMPERATURES

| Animal Size | Temperature (° C.) | *f*-Value |
|---|---|---|
| Large | 16 | <1.0 |
| | 24 | <1.0 |
| | 30 | 1.6 |
| | 35 | 2.7 |
| Mid | 30 | <1.0 |
| | 35 | 2.5 |
| Small | 30 | <1.0 |
| | 35 | 2.1 |
| All sizes summed | 35 | 4.0* |

are notably closer to the 5 per cent level of significance than either of the two previous interactions mentioned. It would be presumptuous to conclude that the saturation-temperature interaction between saturation and temperature represented "near significance" or the like. However, statistical significance is not necessarily identical with biological significance; in the following section data will be presented to show that temperature and saturation do, in fact, have

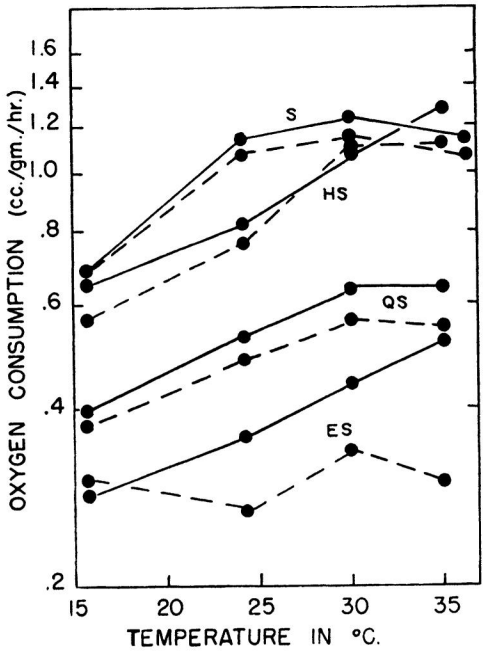


FIG. 5.—Comparison between large *O. immunis* (——) and large *O. nais* (- - -) of the relation between oxygen consumption and temperature (° C.). Oxygen concentration is represented by *S*, saturated; *HS*, one-half saturated; *QS*, one-quarter saturated; *ES*, one-eighth saturated.

a cumulative effect, which lends support to the idea that the interaction values have more meaning than simple statistical non-significance.

## COMPARISON OF RESPONSES OF SPECIES TO THE VARIANTS

Differences in oxygen-consumption rates between the species were tested statistically by analysis of variance. By this method it was possible to test the significance of the entire curve of one species against the comparable curve of the other species, either at a given oxygen-saturation level (Table 4) or at a given temperature (Table 5).

TEMPERATURE

A comparison of the effect of temperature on the metabolic rate of large animals of both species (Fig. 5) shows that essentially no significant differences appear at the saturated and one-half saturated levels. At these two saturations, however, *O. nais* does have a lower metabolic rate in seven out of eight comparable pairs of points. At one-quarter saturation the *R/T* curves are clearly separated, and the differences between the species are statistically significant. These differences increase as temperature increases.

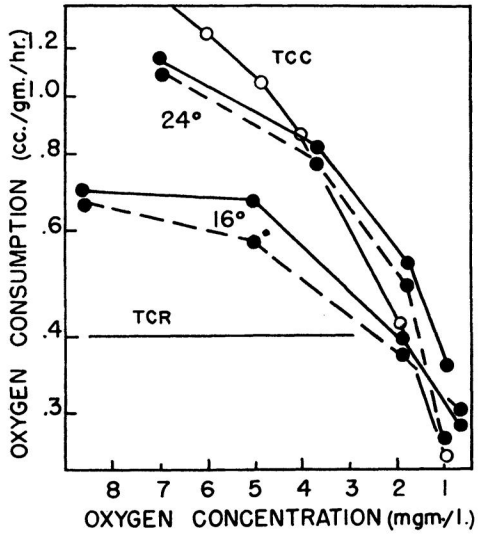


FIG. 6.—Comparison between large *O. immunis* (——) and large *O. nais* (- - -) of the relation between oxygen consumption and oxygen saturation at 16° and 24° C. *TCR* indicates the theoretical curve of a regulator; *TCC* indicates the theoretical curve of a conformer.

The effect of temperature on the oxygen consumption of the two species increases as saturation decreases. *O. immunis* increases its metabolic rate with increased temperature at one-eighth saturation, but *O. nais* is unable to do the same, remaining essentially at the same metabolic level through a 19° C. increase. The mid animals evidence less of a consistent trend than do large animals. Although *O. immunis* had a consistently higher metabolic rate than *O. nais* at 35° C. at all saturations, no statistical significance was obtained between the curves of the species at any saturation level. The small animals, however, do show correspondence to the results seen in the large animals. The curves for saturated and one-half saturated conditions are consistent with those of the other two sizes of animals in that they do not show any significant species differences. The difference between the metabolic rate of both species at one-quarter saturation is significant, being similar to the curves of the large animals at that saturation (Fig. 5). The difference between the two curves increases as temperature increases.

OXYGEN SATURATION

A comparison of the metabolic responses of large animals to changes in the level of oxygen saturation of both species demonstrates that the rate/oxygen concentration (*R/C*) curves of the species are consistently different (Figs. 6, 7, and 8). Thirteen of fifteen different pairs of points plotted in these figures show *O. immunis* to have a metabolic rate higher than that of *O. nais*. Comparison of the *R/C* curves of Figures 6 to 8 with the *R/T* curves of Figure 5 indicates that differences between the species appear considerably greater when data are plotted on *R/T* coordinates. Analysis of variance bears out this observation (Tables 4 and 5), because at none of the temperatures tested does the *R/C* curve of *O. immunis* reveal a statistically significant difference from the comparable curve of *O. nais* at that temperature. If, however, the *R/C* curves of all three sizes at 35° C. are summed, the differences between the species are significant. The implicit conclusion is

that the effect of temperature is greater than that of oxygen saturation in demonstrating a physiological difference between species.

The 16° C. curves of the two species (Fig. 6) reveal that *O. nais* tends toward conformity earlier than *O. immunis* as oxygen concentration drops but does not show any difference from *O. immunis* at one-eighth saturation. The curves for 24° C. show a slight but consistent difference between species, with *O. nais* having the lower rate. The species differences are largest at lowest oxygen saturations. The curves of the metabolic rate of the large animals at 30° C. (Fig. 7) are not completely consistent but, again, do show some of the same species differences at the low saturations. The greatest and most consistent separation

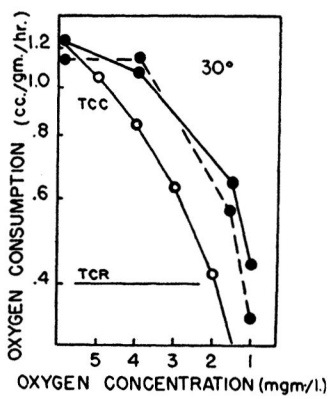


FIG. 7.—Comparison between large *O. immunis* (——) and large *O. nais* (- - -) of the relation between oxygen consumption and oxygen saturation at 30° C. *TCR* indicates the theoretical curve of a regulator; *TCC* indicates the theoretical curve of a conformer.

of metabolic rates among the four temperatures is seen in the curves at 35° C. (Fig. 8). At one-half and one-quarter saturations the separation is moderate, but at one-eighth saturation there develops a greater species difference than is seen under any other set of conditions.

The *R/C* curves of the metabolic rate of both species have a clockwise rotation from low to high temperatures around a point at low concentrations, indicating a steady loss of regulatory powers.

The *TCC* and the *TCR* are plotted in each figure. As previously suggested, any curve that has a slope between the

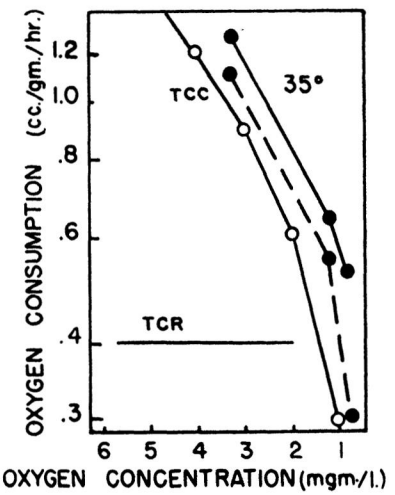


FIG. 8.—Comparison between large *O. immunis* (——) and large *O. nais* (- - -) of the relation between oxygen consumption and oxygen saturation at 35° C. *TCR* indicates the theoretical curve of a regulator; *TCC* indicates the theoretical curve of a conformer.

angles of the *TCC* and the *TCR* indicates an intermediate state of regulation. It is reasonable, then, that if the slope of a curve is more vertically pitched than that of the *TCC*, it must indicate a state of metabolism that is functioning at an even lower rate than that of a conformer. In other words, the ability of the organism to maintain itself metabolically must be breaking down.

The curves of the oxygen consumption of the large animals at 24°, 30°, and 35° C. show such a condition. At temperatures of 24° and 30° C., the curve from full to one-half saturation indicates that the organisms are partially regulating. From one-half to one-quarter satura-

tion, at all three temperatures, the curves indicate complete conformity. From the one-quarter to one-eighth saturation at all three temperatures, the curve of *O. nais* has a distinctly steeper slope than the *TCC*, indicating that *O. nais* is incurring some sort of metabolic breakdown. *O. immunis* is apparently able to withstand a state of low oxygen concentration more successfully. Nothing new may be derived from the *R/C* curves of mid and small animals, except to substantiate that the clearest development of a metabolic separation of the two species occurs at 35° C.

### BODY WEIGHT

Comparison of species in respect to the relation of metabolism to body weight is shown in Figure 4. Generally a difference between the two species occurs at all temperatures, exceptions being the rates of oxygen consumption of the small animals at 16° and 30° C. In cases of clear separation, it is *O. immunis* that has a higher metabolic rate in all sizes of animals. Furthermore, there is an indication that the differences in metabolic rate are greater and more consistent as body weight increases. Previous data have shown that under more severe experimental stress, greater species differences are observed. From Figure 4 it follows that the large animals, of the three size groups, are the most critically affected by these stresses. This effect is, in part, due to the greater absolute amounts of oxygen needed by the large animals that reduce the oxygen concentration lower in the cylinder and force the large crayfish to respire at that lower concentration. Helff (1928), using a different method, also found that the large animals lose regulatory powers at higher saturations than do small animals of the same species. Perhaps Hiestand's (1931) criticism is valid here, in that the oxygen level is being lowered faster by large animals, giving them less time to initiate regulatory activity, with the consequent necessity of conforming sooner.

### ECOLOGY OF THE SPECIES

From early reports to the present time, *O. immunis* consistently has been found in habitats such as intermittent roadside ditches, muddy ponds, and slow, muddy streams (Forbes, 1876; Harris, 1903; Tack, 1941; Williams and Leonard, 1952). Bovbjerg (1952) reports *O. immunis* in the area of Chicago to be one of five species of crayfish inhabiting "permanent woodland and open ponds" in significant numbers. There are no reports at hand of the occurrence of this species in larger, clearer, or swifter bodies of water, such as large lakes or rivers. During the summer of 1959, *O. immunis* was found in only one location in Douglas County, two miles south, and three-quarters of a mile east of Lawrence, in the area locally known as Haskell Bottoms. The occurrence of the crayfish was in a roadside ditch flanked by abundant vegetation. Twice during the summer of 1959, the ditch became completely dry for periods of nearly a week. Average width was six to eight feet, and the water was never over eighteen inches deep. Water was mostly runoff from adjacent cultivated fields and the road.

Harris (1902) reported that he had never taken an *O. immunis* in a running stream. *O. immunis* is a burrower and chimney-builder. Its distribution in Kansas includes only the northeast one-eighth of the state, about fourteen counties; the range of the species (Creaser, 1933) extends from Massachusetts to Wyoming and from Tennessee to upper Wisconsin and Ontario, Canada.

*O. nais* apparently prefers ponds, lakes, and streams with open water. It has been reported both from slow and

from swift streams (Harris, 1902; Creaser and Ortenberger, 1933). The localities from which *O. nais* were obtained were all small, flowing streams, up to ten feet wide, with an average depth of eighteen inches, with deeper holes up to four feet. Some streams were choked and flanked by heavy vegetation; others were nearly devoid of any flora. In all streams the rate of flow was moderate to slow.

*O. nais* is reported (Harris, 1903) to be a burrower in mud and clay banks, but not a chimney-builder. This species is uncommon in very muddy, intermittent ditches, ponds, or in shady streams (Williams and Leonard, 1952). *O nais* is found throughout Kansas; its total range includes the Great Plains and Ozark regions from Louisiana to Manitoba, Canada, and from Colorado eastward to the Mississippi River (Creaser and Ortenburger, 1938).

Regular seinings from May, 1959, to September, 1959, in the ditches containing the *O. immunis* population yielded only one large *O. nais* and several *Procambarus gracilis*; never during this time were any *O. immunis* taken in streams and lakes.

In habitats somewhat similar to those observed locally for *O. immunis* and *O. nais*, Bovbjerg (1952) reported three notable differences between temporary ponds and streams he studied. First, the water temperature of the ponds is generally higher than that of the streams. Second, dissolved oxygen content in the ponds is usually, but not always, lower and is much more variable than in streams. Finally, the ponds are temporary, having no water in them for considerable periods of time during the latter half of the year.

The water temperatures and the concentrations of dissolved oxygen in the local habitats during the summer of 1959 (Table 6) suggest that the three differences cited above are also, by and large, valid differences between the local habitats of *O. immunis* and *O. nais*. The habitat of *O. immunis* was recorded at a maximum temperature of 36° C., temperatures of 30°–35° C. being common. *O. nais*, in the creeks, had a somewhat cooler habitat, the highest recorded temperature of the water being 32° C., while readings 26° to 30° C. were common. Readings were usually taken in the afternoon. The temperature in the crayfish burrows of both habitats at a depth of twelve to eighteen inches below the burrow mouth was 5°–7° C. below that of open water (Table 6).

Dissolved oxygen concentration recorded in the habitat of *O. immunis*

TABLE 6

DISSOLVED OXYGEN CONTENT OF WATER, TEMPERATURE OF OPEN WATER, AND TEMPERATURE OF WATER IN BURROWS

| | Ditch Habitat (*O. immunis*) | | | Creek Habitat (*O. nais*) | | |
|---|---|---|---|---|---|---|
| Date (1959) | $O_2$ (mg/l) | Temp. (° C.) Open $H_2O$ | Temp. (° C.) Burrow | $O_2$ (mg/l) | Temp. (° C.) Open $H_2O$ | Temp. (° C.) Burrow |
| June 19.... | 0.3 | 29.0 | .... | 5.35 | 26.5 | .... |
| 26.... | no water | | .... | .... | 31.0 | .... |
| July 5.... | no water | | .... | 5.24 | 29.5 | .... |
| 12.... | .... | .... | .... | 4.30 | 23.5 | .... |
| 19.... | 3.7 | 23.5 | .... | 3.30 | 22.0 | .... |
| 25.... | 6.4 | 33.0 | .... | 8.12 | 27.0 | .... |
| Aug. 3.... | 8.0 | 36.0 | .... | 5.40 | 32.0 | .... |
| 5.... | 6.5 | 31.5 | .... | 6.40 | 29.0 | .... |
| 10.... | 7.6 | 32.5 | 26.5 | 7.10 | 27.5 | 22.5 |
| 18.... | 7.2 | 31.5 | 25.0 | 6.84 | 27.5 | 25.0 |

ranged from 0.3 to 8.00 mg/l. In the samples taken from the habitat of *O. immunis*, only one reading was below 50 per cent saturation, that being the 0.3 mg/l cited above (about 4 per cent of saturation). Walshe (1948) states that oxygen tension in water in a certain ditch he studied dropped from 92 mm. Hg to a minimum of 3 mm. Hg (1.9 per cent saturation) at night, further substantiating that the oxygen level can actually drop periodically to the low concentration found in the ditches inhabited by *O. immunis*. Park, Gregg, and Lutherman (1940) and Krogh (1941) suggest that the type of differences in oxygen concentration between ponds and streams which Bovbjerg (1952) found may well be taken as a general principle.

## DISCUSSION

The justification of correlations of experimental results with ecological data depends greatly on the extent to which the experimental conditions simulate selected factors of the natural environment.

The experimental levels of oxygen concentration ranged from 0.79 to 8.76 mg/l, compared with a recorded ecological range of 0.3–8.12 mg/l; in terms of oxygen, natural conditions may be even more severe than these experimental conditions.

Experimental temperatures ranged 16°–35° C. Natural habitats ranged in temperature from 22.5° C. in a burrow in the stream habitat to 36° C. in the ditch habitat. Although primary interest is placed on the maximum temperature, the range in ecological temperature is comparable to the experimental range. The time allowed for acclimation to temperature changes under experimental conditions was usually one and one-half to two days for a change of 5°–8° C.; and the conditions of experimental acclimation are presumed to be no more extreme than those occurring in natural situations.

The acclimation time for responses to changing oxygen saturation is more difficult to assess. Van Weel, Randall, and Takata (1954) suggests that acclimation to this factor is accomplished in thirty minutes. Hiestand (1931) has shown that *C. virilis* will continue to regulate longer if brought into an oxygen-deficient environment slowly over a period of hours. If the crayfish are put directly into low oxygen concentrations, they will not be able to regulate. There is no reason to believe that the changes of oxygen concentration in the environment do occur quickly. It is likely that acclimation to this stress is accomplished over a period of time longer than that allowed for acclimation in these experiments. Hence these results should be interpreted as responses to oxygen stress under acute or semiacclimated conditions.

Both species can be identified with those organisms which have the capacity to regulate their internal state under certain conditions, but under conditions of increasing stress, both species tend to lose the power of regulation. For both species it is apparent that the greatest stress on individuals occurs at times when temperatures are highest and oxygen saturations are lowest. Under conditions of greatest stress for both species, the greatest interspecific differences in oxygen consumption occur. Under conditions of great stress, the ability of *O. immunis* to maintain a higher and more nearly regulatory metabolic rate than does *O. nais* under such conditions indicates that *O. immunis* is better adapted to live under environmental conditions of periodic high temperatures and low oxygen saturations. *O. nais* may actually be incapable of sustained life

in the shallow intermittent ditches inhabited by *O. immunis*.

To construct a theory accounting for the ecological distribution of *O. immunis* and *O. nais* on the basis of only two environmental variables is to oversimplify the problem. The preclusion of other factors that may be contributing to the distribution of the populations fails to take into account the fact that the organism is a highly integrated system acted upon by a multiplicity of factors. The organism which has the best adaptation to all these factors will be the most successful. For example, it is certainly reasonable to assume that the ditch habitat's lack of open water for periods of time during the summer months presents to some species of crayfish a limiting situation of some magnitude. There is little possibility that the two species are separated by spatial barriers because the habitats of both species are frequently connected by water flowing from the ditches inhabited by *O. immunis* to a small creek in which *O. nais* is found.

Experimental results do not show a clear physiological basis for the absence of *O. immunis* from the stream habitat. There are two factors which may have some bearing on the question. *O nais*, in the diurnal rhythms studies, was shown to have a daily metabolic rate which was 10 per cent higher than that of *O. immunis*. The higher metabolic rate, coupled with the larger adult size of *O. nais*, might give it the adaptive advantage necessary to keep *O. immunis* from becoming established in the creeks and rivers, if interspecific competition may be assumed.

## SUMMARY

1. Factorial analysis indicates that the effect of size on oxygen consumption of *Orconectes immunis* and *O. nais* is significant; the effect of both temperature and oxygen saturation on the oxygen consumption is highly significant. Interactions of the three variants are not statistically significant; however, the data show a clear indication that the cumulative effect of increased temperature and lowered oxygen concentration produces a greater stress than either factor acting independently.

2. The metabolic response to temperature change is a uniform increase of oxygen consumption with increasing temperatures up to approximately 36° C. The mean $Q_{10}$ for *O. immunis* was 1.42; that of *O. nais* was similar.

3. The response to decreasing oxygen saturations is a corresponding decrease in metabolic rate. However, both species were partial regulators of their internal states under moderate experimental stresses.

4. There is a decrease in metabolic rate with increasing body weight.

5. *O. immunis* and *O. nais* do not have a statistically different rate of oxygen consumption under experimental conditions of moderate temperatures and moderate oxygen concentrations. However, the two species have significantly different metabolic rates under stresses of high temperatures and low oxygen saturations. The difference in rate appears to be due to failure of *O. nais* to regulate as well as does *O. immunis* under extreme conditions.

Correlations of experimental data with ecological conditions suggest that the inability of *O. nais* to regulate as well as *O. immunis* may bar it from the road side ditch habitat in which *O. immunis* lives.

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